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EDITORIAL

SELENIUM THE STRANGE

ELSEWHERE in this issue is printed an original article contributed by a former student of this institution, now pursuing special researches in Germany. Dr. Strock, the author, is studying the strange element selenium, sulphur's erratic sister, which for some time past has been suspected of leaving the soil through the greens and poisoning the grazing cattle.

Like sulphur, this substance—born of volcanic distress—is a red-loving radical element. Capable of several physical forms, its properties are paradoxical and queer. Toxic and tonic, electric and static, colorless and colorful, its compounds run a giddy gamut of properties and uses.

The Arthur D. Little Industrial Bulletin, a lively little periodical, appearing all too infrequently, is responsible for the remainder of this timely bit of writing pertinent to selenium's erratic but useful career.

According to that journal, the recent publication on "The Selenium Problem," by Dr. Henry G. Knight, of the United States Bureau of Chemistry and Soils, brings to the fore a chemical element with several unique properties. The "problem" concerns the occurrence of selenium in certain soils, particularly in parts of such semi-arid states as South Dakota and Wyoming, which causes poisoning of cattle and horses feeding on certain types of plants growing thereon. The so-called "blind staggers" or "alkali disease" of animals has been found to be caused by the ingestion of appreciable amounts of selenium, which spreads through all parts of the animal, but concentrates most in the liver. The highest concentrations of selenium are found in certain weeds not often eaten by stock, but can occur in dangerous amounts in particular species of leguminous plants which

are eaten. Fortunately, the wheat raised on seleniferous soils does not run high in selenium, and that, with the modern milling technique which demands much blending, makes the problem one of only minor direct concern to human beings. Feed grasses, low in selenium, can be raised on seleniferous soil which may be safely fed to the animals. To be on the safe side, however, the Bureau has made provisional recommendations for the withdrawal from production of some 50,000 acres of land. The problem is world wide in its scope, and doubtless also needs consideration in Canada, Australia, the Argentine, and other countries with semi-arid wheat-raising areas.

Selenium compounds, taken internally or absorbed through the skin, cause the whole body to take on a most vile and offensive odor, suggesting horseradish, garlic and brass all at once. It takes real courage for chemists who realize the possibilities to work with the selenites and selenates, for a single familiarity may scent one's perspiration and breath for a month. The skunk might have a research job done by the chemist, using selenium, to *increase* his "Schrecklichkeit."

Selenium is the next higher member above sulfur in the sulfur group, and it possesses many of the chemical and physical properties of sulfur. For instance, it may be used alone in vulcanizing rubber. Used in small proportions, in conjunction with sulfur, it imparts to rubber a wear resistance, not given by sulfur alone, which has been applied to auto tires and to extension lamp cords which are expected to be dragged over concrete floors. Strangely enough, when four parts of selenium are combined with one part of sulfur, this completely inorganic mixture is lastingly flexible, with physical properties intermediate between those of lead and those of rubber. Selenium, sulfur, and rubber have much in common physically besides working well together.

A most remarkable property of selenium is its ability to suppress the burning of rubber. As little as a pound of the powdered element adhering to the rubber insulation, but under the braid, of a mile of wire will make this wire flame-proof. This patented use is especially applicable to telephone exchange wire. A slight smudge of selenium near the bottom of a match made of sulfur (used in testing for ammonia leaks) causes the sulfur flame to go out at once when the smudge is reached. This property is all the more remarkable when it is realized that selenium itself is sufficiently inflammable to burn

with a flame under certain conditions. Whether by way of paradox or irony, a patent was issued recently for the use of a mixture of powdered selenium and powdered metallic tin, for the ignition mixture in blasting caps.

Sulfur is definitely non-metallic, and in fact is one of the best electrical insulators. Selenium may be either metallic or non-metallic, whereas the next higher member, tellurium, is definitely metallic in appearance and in electrical conductivity. Selenium conducts electricity better when it is illuminated than when it is in the dark. This property has been put to use in making one type of the "electric eye," and some of the more recently improved selenium photo cells are used in relays for turning on lights when darkness comes, for light signalling, and even for television.

Selenium finds application in the glass industry, both to produce the red color of automobile tail lights and other red glasses, and to serve as a "bleach" to neutralize the green of glass, or even to throw it a little toward the pink side and for making mirrors which make one's reflection even more optimistic than the original.

Which in these days is quite a contribution to the public good.

Vitamin D and Rheumatism

Rickets-preventing Vitamin D is of great benefit in the treatment of arthritis or rheumatism, as it is sometimes called, Dr. C. I. Reed, of the University of Illinois College of Medicine, told members of the American Physiological Society.

Seventy out of one hundred arthritis patients treated this way by himself and associates, Drs. M. L. Hathaway and H. C. Struck, were definitely helped and some apparently cured.

The vitamin was given in the form of concentrated viosterol and enormous doses were used. While three thousand units is the standard dose for rickets treatment, Dr. Reed used one million units and in some cases three million to treat the arthritis patients. All kinds of arthritis except that due to gonorrhea were helped.

ORIGINAL ARTICLES

THE DISTRIBUTION OF SELENIUM IN NATURE

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Introduction

DURING the past winter the author has had the opportunity of analyzing a large variety of geological materials for their selenium content. The results of these analyses have been published in a paper on the Geochemistry of Selenium together with *V. M. Goldschmidt.*(1) In the present paper that portion of this new data on the distribution of selenium in nature is summarized, which is of interest to other workers on the selenium problem, especially in the field of Biology and Agriculture. For the detailed analyses and discussion of the distribution of selenium in purely mineralogical materials, the original paper must be consulted.

General Remarks

The discovery of selenium in the soils of certain areas of the north central Great Plains of the United States, has led to the assumption that this element is the cause of a serious animal disturbance endemic to this region. This has stimulated investigation not only on the occurrence of selenium in plants, soils, and grains of the affected areas, but has initiated studies on the dependence of plant growth in media of known selenium content. The pathological effect on various animals of grains grown in these affected areas has also been studied by systematic feeding experiments.

While it is not yet firmly established that selenium is the specific cause of the so-called "alkali disease" most of the available evidence supports this assumption. The mere probability, however, that a highly toxic effect may be produced by a foodstuff which has absorbed selenium during some phase of its life cycle, demands that the problem be given careful consideration by agriculturists, botanists, dietitians, pathologists and pharmacologists.

The first and most important question which any of these investigators should consider, is the source of the selenium present. Whether the disturbing selenium is present in plant or in animal products, the soil and surface waters on which the original plants were grown, or on which the animals grazed, would in general be suspected as being the source of the selenium.

With the exception of a possible contamination of the soil by selenium-containing fertilizers and disinfectants, the natural sources of selenium in soils are their parent rocks and in special cases circulating ground water and waters rising to the surface by capillary action. Since soils are the residues and metamorphic products of the primary phases of the earth's crust which have survived all the geological processes of erosion, their subsequent deposition, elevation, repeated re-erosion and re-deposition, their content will depend upon the manner in which this element takes part in these various geochemical processes, as well as upon the amount of selenium present in the primary materials of the earth's cust.

Previously Known Facts on the Distribution of Selenium

Previous quantitative knowledge on these points is confined chiefly to the following experimental results:

1. *V. M. Goldschmidt* and *O. Hefter* (2) have shown, that if the sulfates from the central German marine salt deposits contain selenium at all, it is much less than 1 part selenium to 500,000 parts by weight of sulfur. The ratio of sulfur to selenium in these marine evaporates is therefore at least 100 times greater than in the primary sulfides, so that a separation of selenium from the sulfur has occurred somewhere in the erosion and sedimentation cycle.

2. Considerable quantities of selenium are known to occur with sulfur of volcanic origin. *C. Doelter* (3) quotes an analysis of natural selenium-sulfur from Hawaii which contained 94.82 per cent. S and 5.18 per cent. Se, corresponding to a Se:S ratio of 1:19. Sulfur of volcanic origin results from the pneumatolytic reaction of H_2S with SO_2, O_2, or H_2O, according to *W. Lindgren,*(4) so that if selenium be found in volcanic sulfur it must have been contained in the original volcanic gases.

On the other hand the large occurrence of sulfur in sedimentary rocks are either free from selenium (Louisiana) or contain only traces of this element (Sicily).

3. Due to their economic importance, in the manufacture of sulfuric acid, the selenium content is well known for those iron and copper sulfides whose origin is closely associated with magmatic rocks. The average selenium content of these sulfides is close to 0.005 per cent. corresponding to a selenium-sulfur ratio of about 1 :10.000. When such ores are roasted and the gas converted to sulfuric acid by the lead chamber process, the selenium accumulates in the lead chamber sludge, which constitutes one commercial source of selenium. Selenium is also reclaimed from the residues formed in the electrolytic refining of copper.

4. The remaining pure mineralogical data concerning the distribution of selenium in nature consisted of a few scattered occurrences of some rare selenide minerals in a few silver deposits of uncertain geological origin, (5) for example, at Cerro de Cacheuta, Argentina, and in the region of Guanajuato, Mexico. Other selenides occur in various localities of the Harz Mountains of Germany.

5. The workers of the Bureau of Soils in Washington have recently published the results of numerous selenium determinations. *W. O. Robinson, H. C. Dudley, K. T. Williams* and *Horace G. Byers* (6) and *K. T. Williams* and *H. G. Byers* (7) have reported the following data on selenium. In soil derived from the Pierre shales they found as much as 32 p.p.m (0.0032%) selenium, while in the shales themselves they observed selenium from traces up to 103 p.p.m (0.0103%). A pyrite nodule from this shale contains 205 p.p.m (0.0205%) and gypsum crystals from the same formation contained up to 15 p.p.m (0.0015%) selenium, while shallow well water in the same region along with its high sulfur content contained 0.07 p.p.m selenium.

In a series of United States pyrites the same authors found selenium contents ranging from 0 to 250 p.p.m which gave an average of 59 p.p.m. They published no sulfur determination on the same material, but a probable value of 40 per cent. sulfur for the pyrites leads to an average Se:S ratio of 1 :6800. The Se:S ratio for the Pierre pyrite would be 1 :2000 and for the gypsum 1 :15000. In some Alabama shales they found a Se content ranging from 0.15 to 0.6 p.p.m. The failure of the authors to publish the exact geological occurrence of many of their materials, especially the pyrites in addition to the lack of sulfur determinations on the same material, makes it impossible to use their accurately determined selenium values

for drawing any conclusions concerning the distribution of selenium in the various phases of geological processes.

6. The values of *I. Noddack* and *W. Noddack* (8) for the absolute frequency of the chemical elements as determined by "precision analyses" on average samples of the entire earth's crust and of meteorites, do not reveal the manner in which selenium is distributed in the individual materials of the earth, as thus far only average data have been published. For the earth's crust as a whole they give absolute values for sulfur and selenium whose ratios are Se:S 1:1600 and for meteorites 1:2400. A recalculation of their "atom frequencies" gives for the earth's crust 0.0000303 per cent. by weight and for meteorites 0.00084 per cent. selenium.

New Determinations on the Distribution of Selenium

In order to collect additional information concerning the amount of selenium in geological materials of known origin, a series of selected specimens representing the principal phases of nature's primary processes and of the secondary erosion and sedimentation cycle, were analyzed for this element.

This work has been done in the Mineralogical Institute of the University of Göttingen, Germany, on material supplied by *Prof. V. M. Goldschmidt* from the Institute's collection of the world's more important geological and mineralogical formations of certain origin.

In products of magmatic origin: As has been mentioned by *Goldschmidt* and *Hefter* (9) the sulfur in magmatic rocks is bound almost exclusively as metallic sulfides, and since the radius of the divalent negative sulfur (1.74 Å) and selenium (1.91 Å) ion are very similar, selenium is frequently found occurring in isomorphic mixtures with sulfide minerals. As a representative of one of the three primary differentiation phases of meteorites (metallic, sulfide, and silicate) two meteorite troilites were analyzed. In troilite from the Corrizatillo, Chile, meteorite 0.0132 per cent. Se was found, and on the same sample 37.48 per cent. S, which furnishes a Se:S ratio 1:2840. Almost identical results were obtained from Canyon Diablo troilite.

The corresponding sulfide phase of the initial magmatic differentiation in the earth is inaccessible but many sulfide bodies are known which are formed by the fractional crystallization of smaller bodies of intruded basic magmas.(10) To these belong the pyrrhotite-pentland-

ite sulfide ores. The average selenium content of a series of these from various localities was found to be 0.005 per cent., which furnishes a ratio between sulfur and selenium of 4000-5000. These results are in agreement with the already known selenium content of those iron-copper sulfides which are found associated with magmatic rocks.

In molybdenite from Knaben, Norway, was found 0.019 per cent. Se (190 p.p.m) and 34.85 per cent. S corresponding to a Se:S ratio 1:1834. In stannite from Cornwall, England, was found 0.0044 per cent. Se which furnishes a Se:S ratio 1:6170. These two minerals are representatives of magmatic pneumatolytic processes, and are further proof that selenium accompanies sulfur in magmatic gases. Up until the present volcanic sulfur has been the only quantitative mineralogical proof of this fact.

A series of sulfides of hydrothermal origin were also analyzed. Their selenium content was very low—yielding a Se:S ratio of about 1:300,000. These analyses throw some light upon the distribution of selenium in sulfides which are definitely associated with the differentiation and cooling of the primary magma. Selenium is richer in the primary sulfide phase of the first differentiation of the earth's magma, i. e. in the sulfides whose differentiation has closely followed that of the metallic iron or has separated from the magmatic iron on further cooling. On the other hand the sulfides which were originally dissolved in the primary silicate phase but later settled out due to fractional crystallization of the silicate magma on further cooling contain two to three times less selenium (nickeliferous pyrrhotites, etc.). Pneumatolytic sulfides which are formed from the gases derived from the magma, as for example molybdenite, contained as much selenium and have a Se:S ratio equal to that of the sulfides which have separated for the metallic iron phase, i. e. troilite. The new data also shows that very little selenium follows the hot aqueous solutions which issue from the magma at the last stages of its cooling.

In the erosion cycle: The solidified silicate magma in all of its related forms is sooner or later exposed to erosion. It has been proven that the behavior of selenium in the erosion cycle is not so simple as earlier supposed. The most ideal behavior of selenium after it has been dissolved from volcanic rocks is exemplified from the until now unknown presence of selenium in the "caliche" saltpeter beds of Chile.

According to *W. Lindgren* (11) the "caliche" beds are a reddish-brown sandy gravel cemented with salts which have been leached from the extensive tertiary volcanic rocks of the region under unusual climatic conditions. They occur in the Atacama Desert situated in the valley which lies between the Coast Range and the Andes in Northern Chile. The rainfall in this district is small and that which does fall, never leaves the region. (12) Here exist the strongest oxidizing conditions under which any known weathering erosion and sedimentation is taking place, as shown by the state of oxidation of the compounds formed: nitrogen is converted to nitrates, sulfur to sulfates, chlorine partially to perchlorate, chromium to chromates, and iodine to iodates. *V. M. Goldschmidt* and *O. Hefter* (13) have called attention to the fact that the oxidation-potential of the usual erosion processes is sufficient to oxidize sulfides to sulfates, that is to a usually soluble form, but that a much higher oxidation-potential is necessary to oxidize selenides to a corresponding state. Concerning the oxidation-potential of selenic acid, see the paper by *M. S. Sherill* and *E. F. Izard.* (14) One would expect that in the "caliche" where the leaching occurred under strong oxidizing conditions, and where the resulting solutions have traveled only a relatively short distance before rising to the surface and evaporating, that selenium would accompany sulfur in its original ratio and be in its higher state of oxidation like all the other constituents present. This is actually the case, as was determined by analyzing a mixture of 14 various samples of the "caliche". There was found 0.00051 per cent. selenium and 4.00 per cent. sulfur which gives a Se:S ratio of 1:7850. All of this selenium is present in a very soluble form.

The products of rock weathering, erosion, and sedimentation are in general a solid residue and a solution of the soluble constituents most of which may eventually reach the seas. In the course of time some selenium must have reached the ocean, derived both from the eroding of igneous rocks with their accompanying sulfide masses and from volcanic exhalates. In order to trace the movement of selenium in the erosion cycle it was therefore necessary to determine how much of this element is now present in sea water. The sulfur content of sea water is known to be 0.088 per cent. (15) If all of this sulfur has been derived from the erosion of magmatic rocks, with their included selenium-containing sulfides, and if the original selenium of these rocks has accompanied the sulfur and both elements have re-

mained unseparated from one another until the present time, one liter of sea water should contain about 0.2 mg. Se. An analysis of sea water from Helgoland revealed the presence of definite amounts of selenium, but the amount present is only 0.004 mg. per liter or fifty times less than that, which ought to have been added to the sea by the erosion of primary rocks.

It is therefore necessary to find where the major portion of the selenium remains.

V. M. Goldschmidt and *Cl. Peters* (16) have recently called attention to a similar removal of arsenic from the sea, for of the 3 mg. per liter arsenic carried to the sea in the course of geological time only 0.02 mg. per liter remains. This "Entgiftung" of the sea has been shown to have resulted from a process well known in Pharmacy, namely the absorption of arsenic by freshly precipitated ferric hydroxide. Accordingly all marine sediments in which freshly precipitated iron hydroxide was accumulated during their deposition, contain as much as 0.1 per cent. As, this includes many commercially important sedimentary iron ore deposits. The high arsenic content of these marine iron ores has been chemically determined by *L. W. Strock* (17) as well as spectrographically by *Goldschmidt* and *Peters*. Reference should be made to the work by *G. Lockemann* (18) upon the absorption of arsenic by ferric hydroxide. To test the probable absorption of selenium by ferric hydroxide, 0.250 Gm. iron was precipitated as Fe $(OH)_3$ from 500 cc. of a solution containing 1.0 mg. selenium as Na_2SeO_3. The precipitate was filtered off and analyzed —the selenium was found to have been quantitatively absorbed by the $Fe(OH)_3$.(19) To 500 cc. sea water were added 0.05 mg. Se as Na_2SeO_3 and ferric hydroxide was precipitated from the solution: the selenium was quantitatively absorbed by the hydroxide. This simple experiment therefore offers a simple explanation of how at least a portion of the selenium is removed from the sea.

A series of sedimentary iron ores was then analyzed for their selenium content. Selenium was found in all of them in amounts ranging from 0.0001 per cent. to 0.0005 per cent., and on the basis of sulfur analyses on the same specimen they furnish a Se:S ratio of 1:250 to 1:1000. An analysis of a manganese nodule dragged from the bottom of the Atlantic Ocean by the Challenger Expedition gave the same result. The process of absorption therefore, removes selenium from the seas and concentrates it in iron-rich marine sedi-

ments with respect to sulfur by a factor of 20; as compared with the ratio of these two elements in magmatic sulfides.

The strong absorptive power of freshly precipitated ferric hydroxide for selenium is therefore the explanation of the observation made by *K. T. Williams* and *H. C. Byers* on a weathering clay bed near Bay Springs, Miss. They found, that although weathering produces both water soluble selenium and sulfur, the removal of the former does not keep full pace with the removal of the latter from the limonitic material. They rightly claim, that the 45 p.p.m selenium content of a limonite pseudomorph after pyrite is further evidence of this fact.

Since selenium is being constantly separated from sulfur in sea water, it is to be expected that those sulfates which crystallized out from ancient seas as they dried up should contain only very little or no selenium. This was actually found to be the case for the central German marine salt deposits by *Goldschmidt* and *Hefter.* (20)

Not only is selenium present in rather pure iron sediments, but also in small but determinable amounts in other sediments which of course contain some iron hydroxide. In a specimen of "Culmschiefer", the marine phase of the lower Carboniferous of Germany, was found 0.00001 per cent. Se (0.1 p.p.m) which yields a Se:S ratio 1:9000. Pyrite crystals which form along fractures in this slate were found to be comparatively selenium-rich (0.0026 per cent. Se yielding a Se:S ratio of 1:14000), as compared with other sedimentary pyrites, for example one from a coal bed which contained only 0.0008 per cent. Se, and a pyrite goniatite fossil which contained only 0.0005 per cent. Se.

Reference should be made to the analyses of *E. Minami* (21) on three composite samples of shales which were also made in the Mineralogical Institute here in Göttingen. He obtained the following values:

European paleozoic shales (36 samples) (S 0.32%)	Se 0.00011% (Se:S 1:2900)
Japanese mesozoic shales (10 samples) (S 0.20%)	Se 0.00004% (Se:S 1:5000)
Japanese paleozoic shales (14 samples) (S 0.12%)	Se 0.000024% (Se:S 1:5000)

In the Mansfield "Kupferschiefer" of Central Germany was found 0.001 per cent. Se. Reference has already been made to the selenium content of some Alabama shales published by the workers at the Bureau of Soils, and of the very high Se content of the Pierre shales of South Dakota and Wyoming. While iron hydroxide-rich sediments have derived their selenium from the waters in which they were deposited by the absorption action of $Fe(OH)_3$, it dare not be assumed that all shales and slates have derived all of their selenium in this manner. The "Culmschiefer", the composite samples of shale analyzed by *E. Minami,* and especially the "Kupferschiefer" contain considerable amounts of carbonaceous matter derived from plant remains. It must be remembered that these plants are a possible source of selenium, since certain plants can extract selenium from soils. Perhaps some connection will eventually be found between the selenium-rich Pierre shales and their organic content. Since the Pierre is of marine origin the selenium may have been derived from the draining of "caliche"-rich erosion surfaces forming on the eastern slopes of the then rising Cordillerans. Another possible source is the overlying early tertiary lignite beds, whose possible selenium content may have been leached out during their erosion, and concentrated by absorption in the underlying Pierre shale. Corresponding to conditions in the Atacama Desert today, the leaching of the newly uplifted Cordillerans, and of the igneous rocks intruded at this time on their eastern margins, under rather arid conditions may have formed "caliche" beds on their eastern slopes and lowlands whose early subsequent erosion transferred these salts to the shallow waters in which plant remains (lignite) were being collected, in the early tertiary sea just to the east. Some of these plants themselves may have grown in these salt-rich formations and have carried selenium into the lignite deposits. At any event these uneroded tertiary lignite beds which lie conformably above the upper cretaceous Pierre shale of South Dakota and Wyoming should be analyzed for selenium. It should also be ascertained whether the selenium content of those Pierre shales whose overlying lignite (for example Ludlow member) have not been eroded and leached, is as high as those now exposed and forming selenium-rich "gumbo" soil. The geology of this region is described in the various Bulletins from the Geological Survey, Washington. (22)

On the basis of experimental work of others, shortly to be referred to, plants are known to absorb selenium from the medium in

which they grow. Such selenium-containing plants which eventually accumulate in coal, in lignite beds, shales, or even limestones would add their selenium content to these formations.

In this connection the selenium content of a 4 gram sample of chimney soot investigated, should be mentioned. This amounted to 0.083 mg. corresponding to 0.0021 per cent. This, in connection with the presence of selenium reported by *A. Jorissen* (23) in the ashes from a steam heating plant which used coal from Lüttich, make it highly probable that selenium is accumulated in coal beds, although attempts (by the author) to isolate it from unashed coal have failed. This is further supported by the detection of selenium in coke by *Smith.*(24)

There is certain mineralogical evidence which makes an examination of the large sedimentary calcium carbonate deposits necessary, This work has been commenced but it is not yet completed.

Because of the ability of selenium to be absorbed by plants and freshly precipitated ferric hydroxide, all sedimentary deposits containing considerable amounts of these substances are potential sources of selenium. In this way selenium is removed from the erosion cycle and held until subsequent elevation and reerosion redissolves it and starts it on a new cycle.

The seepage of water into those uplifted sediments may dissolve the selenium and sulfur, and then carry them in solution to greater depths where they are reduced and crystallized out as pyrite along fracture lines in the rock. The concentration of selenium is too small for it to form mineral species of its own, but it remains with the sulfur in the original Se:S ratio as an isomorphous mixture in pyrite, as was found for the Culm slate of Germany. The high selenium content of the pyrite concretions which occur in the gypsum-rich Pierre shales, show that similar reactions are in progress there.

Unless brought in by subsequent magmatic intrusions, the selenium content of crystalline rocks resulting from the dynamic metamorphosis of sediments, such as for example the gneisses about Philadelphia, will be determined by the selenium content of the original sediments. Not until completion of the investigation on more formations of various geological ages and especially on the calcium carbonate ones, will it be possible to make general conclusions concerning the type of sedimentary rocks in which selenium may be expected to be present. Two processes by which selenium may accumulate in rocks by purely sedimentary processes are certain, namely,

absorption from the water by ferric hydroxide and absorption by plants growing in the water or carried in from the land.

The source of selenium in soil is therefore their parent (1) selenium-rich sedimentary rocks (shales, slates, coal or lignite), (2) magmatic rocks with sulfide masses, (3) eroding "caliche" beds, (4) waters rising to the surface by capillary action which have leached any of the other types of formations. It is not yet determined whether limestones and sandstones constitute a potential source of selenium or not.

The analyses discussed above show that the average selenium content of those shales investigated lies between 0.1 and 1.0 parts per million (0.00001 per cent. and 0.0001 per cent.). Special local conditions may have of course caused some shales to become much richer in selenium than others. More extensive co-operative soil investigation and agricultural research will be required to determine if young soils derived from shales containing 1 p.p.m selenium in regions of normal rainfall show any detectable effect on the vegetation which they support or if this vegetation absorbs sufficient selenium to produce toxic effects in animals.

Review of Recent Biological Investigations on Selenium

The various lines of investigation which the discovery of selenium in the soils and vegetation of certain "alkali disease" (25) affected regions of South Dakota, Nebraska, and Wyoming have stimulated, are mentioned in the introduction.

The effect of selenium as Na_2SeO_4 in the soil (and culture solutions) in which wheat was grown (Hard federation wheat—Triticum vulgare Vill.) has been studied by *A. M. Hurd-Karrer* (26) who shows that a chlorosis developed in the plants when 0.1 p.p.m Se was present in soil and that 1 p.p.m Se killed the plants. The author claims that this toxicity was entirely inhibited when 32 p.p.m sulfur were added either in the form of sulfates or elementary sulfur. It is also claimed that the presence of at least ten times as much sulfur as selenium will inhibit the toxicity of the latter. The toxicity of 18 p.p.m Se was inhibited by adding 192 p.p.m sulfur. All these experiments show very definite dependence of the wheat growth on tha selenium content and the Se:S ratio. No sulfur analysis was given for the original Keyport Clay-loan soil used, which may have amounted in some cases to several times the quantity added. The

lower toxicity of added selenium in clay loam soil, as compared with pure quartz sand, is undoubtedly due to an absorption of selenium on the finely divided soil particles, most likely by the organic or colloidal matter. The grain and straw from these plants grow in soil containing as low as 1 P.p.m selenium, too little to visibly injure the plants, were very poisonous to rats and guinea pigs. *W. O. Robinson* (27) reports the presence of 5-12 p.p.m (0.0005-0.0012 per cent.) Se in "poisonous" wheat, as well as 0.009 per cent. Se in a gluten presumably extracted from a "poisonous" wheat, but no sulfur analyses were published so that whether or not this represents an enrichment as compared with sulfur is unknown. *Kurt W. Franke* (28) of South Dakota State College has made systematic feeding experiments using the toxic grains obtained from farmers of South Dakota whose livestock had shown pathological symptoms. These grains were found to be extremely toxic to white rats, and *Franke* has studied the pathological effects which they produced on these rats. He found that the liver seemed to be one of the primary foci of the toxicant (thought to be selenium) for it was atrophied, necrotic and hemorrhagic in varying degrees. In this connection a recent paper by *H. C. Dudley* and *H. G. Byers* (29) is of interest. They report 25 p.p.m Se in the liver of a calf (presumably a selenized one) as compared with no selenium in a normal calf liver. However, they as before, give no sulfur values on the same material. It may however be pointed out that this amount of selenium corresponds to the same concentration in which this element is present in its primeval source in the magmatic sulfides of the earth's crust, which contains 30-50 per cent. sulfur. Since the sulfur content of a normal or pathological liver is far less than 50 per cent., the liver must possess an enormous capacity for absorbing selenium. This fact is of tremendous importance for a geochemical discussion of selenium, and illustrates what an important factor living organisms may be expected to play in concentrating this element. *Franke* (30) also showed that the toxic principle in these grains is extracted with their protein fraction. *K. W. Franke* and *Van R. Potter* (31) have also shown that rats fed on toxic wheat develop strong anemia before their death. *K. W. Franke* and *A. L. Moxon* (32) have demonstrated that protein from toxic grain will not increase the rate of fermentation of a yeast and glucose mixture as contrasted with protein from normal grain.

The serious effect (thought to be due to selenium) produced on the livestock in the affected region are well illustrated in Cir-

cular No. 320, of the U. S. Department of Agriculture. All of these investigations demonstrate that selenium is poisonous to plants and animals, and the potential danger of only minute quantities of this element in soil.

C. B. Gnadinger (33) has shown that the commercial selenium-containing insecticide "Selocide" is an effective spray in combating the red spider. The poisonous action of selenium on plants and animals however makes its use as a general spray very dangerous, as has been pointed out by *E. M. Nelson, A. M. Hurd-Karrer* and *W. O. Robinson.*(34) *O. A. Beath, J. H. Draize, H. F. Eppson, C. S. Gilbert* and *O. C. McCreary* (35) have recently shown that some poisonous two-grooved milk vetches (Astragalus bisculatus and others) have a highly increased toxicity when grown on certain shales among which are the Pierre and other beds of Cretaceous age. This was traced to the presence of 0.1 per cent. in the plants. Animals eating these plants develop a disease known locally as "Blind Staggers" which may be produced artificially by Na_2SeO_3.

In this connection the detection of selenium in plants growing on the banks of a mineral spring at La Roche-Posay, France, by *F. Taboury* (36) should be mentioned. The same author much earlier (37) found 0.2 mg. S per liter in the waters of these springs.

These various investigations serve to show the economic importance of the distribution of selenium in nature, and although selenium must be classed as a rather rare element in spite of its even atomic number 34, its occurrence in rocks and soils in amounts as small as 1 to 10 parts per million may constitute a danger to agriculture.

It is hoped that the work which is now in progress on other sedimentary formations will enable a more complete discussion of selenium in the crust of the earth to be presented shortly.

It is a pleasure to thank *Prof. V. M. Goldschmidt,* who suggested this problem, for his constant stimulating advice and for generously supplying me with specimens from the Institute's collection. I am indebted to *Frl. Binder,* chemical technician, for making the sulfur determinations. I am also grateful to *Dean Dr. Chas. H. LaWall* through whose endeavor the Philadelphia College of Pharmacy and Science granted a stipend which enabled me to continue my work here in Göttingen.

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THE HAND HOMOGENIZER AND ITS USE FOR THE EXTEMPORANEOUS PREPARATION OF PHARMACEUTICAL EMULSIONS

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IN RECENT years there has appeared on the market a small, inexpensive hand homogenizer which has been sold primarily as a kitchen utensil for the purpose of incorporating sweet butter with milk and thus preparing cream.

The efficiency of this apparatus and its simplicity of construction and operation lead the author to investigate its usefulness as a piece of small scale pharmaceutical equipment, especially for the prescription pharmacy.

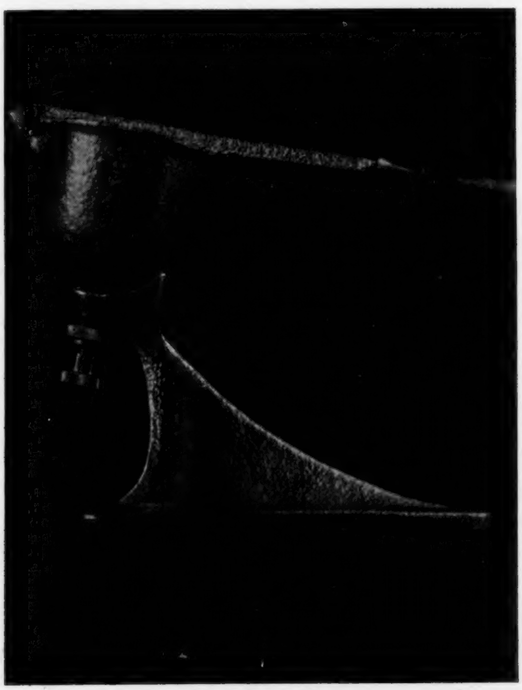

Fig. 1.

In this study the particular device employed was the "Club Aluminum Cream Maker." * A photograph of this device, together with a sectional drawing, appear in Figs. 1 and 2, respectively.

The operation of this instrument is based on the principle of homogenization which is the most desirable method for the preparation of emulsions. The phase to be dispersed is atomized together with the dispersion medium through a fine orifice against a high back-pressure. This results in a very efficient breaking up of the dispersed phase into very small globules which are prevented from subsequent coalescence by the presence of the third phase, the emulsifying agent.

In employing the hand homogenizer, the procedure is quite simple, e. g., in preparing an oil in water emulsion using acacia as the emulsifying agent the method is as follows:

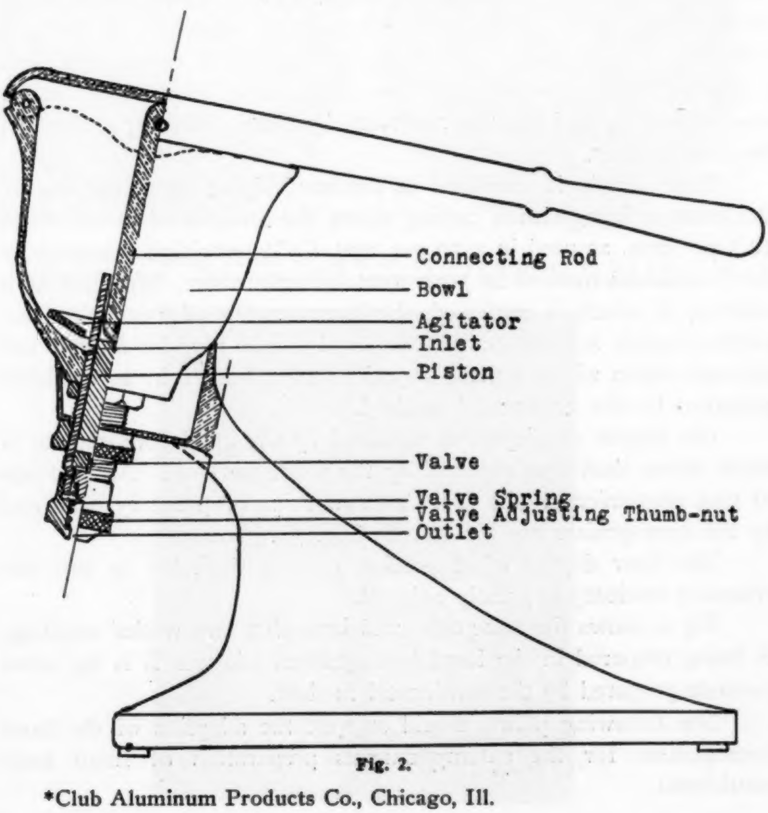

Fig. 2.

*Club Aluminum Products Co., Chicago, Ill.

The acacia is dissolved in the aqueous phase, the correct amount of oil added and then the mixture roughly shaken in a bottle. The mixture is then poured at once into the reservoir of the homogenizer and pumped through the device by a slow but forceful motion on the handle. It is usually advisable to return the emulsion a second time and pump it through to insure perfect dispersion of the oil. At the discharge orifice there is a thumb nut which enables one to adjust the back-pressure against the valve and so increase or decrease the degree of dispersion. For pharmaceutical emulsions, this thumb nut should be screwed up tightly to give the best dispersion.

The homogenizer is very easily cleaned by unscrewing the thumb nut which permits the removal of the valve parts.

Emulsions may be prepared in a very few minutes with this device and without any danger of failure as it is impossible to crack or ruin an emulsion prepared in this manner, regardless of type, if the ratio of ingredients employed is fundamentally sound. In fact, a cracked emulsion which has been ruined by a trituration method may be quickly and entirely recovered by simply running it through the homogenizer.

When acacia is employed as the emulsifying agent the use of the homogenizer permits cutting down the amount of acacia from 12.5 per cent. as used in a 50 per cent. O/W emulsion prepared by the continental method to 5 per cent. Furthermore, inasmuch as a solution of acacia is employed, the large amount of mechanical impurities which are present in powdered acacia may be filtered out through cotton whereas these impurities must remain in an emulsion prepared by the continental method.

The degree of dispersion obtained by the hand homogenizer is much better than that obtained by the usual methods. As evidence of this, photomicrographs of both an emulsion prepared by hand and by the homogenizer are illustrated (Fig. 3).

The finer degree of dispersion is very desirable in that the creaming tendency is greatly reduced.

Fig. 4 shows the same two emulsions after two weeks' standing, A being prepared in the hand homogenizer, whereas B is the same formula prepared by the continental method.

The following points would suggest the adoption of the hand homogenizer for the extemporaneous preparation of small scale emulsions:

I. The efficiency, versatility and ease of operation of this apparatus are outstanding.

II. It provides a saving of materials both in the elimination of waste through cracked emulsions and the reduction in the necessary amount of emulsifying agent.

III. An emulsion prepared by its use possesses a higher degree of dispersion and a much slower rate of creaming than emulsions prepared by the usual trituration methods.

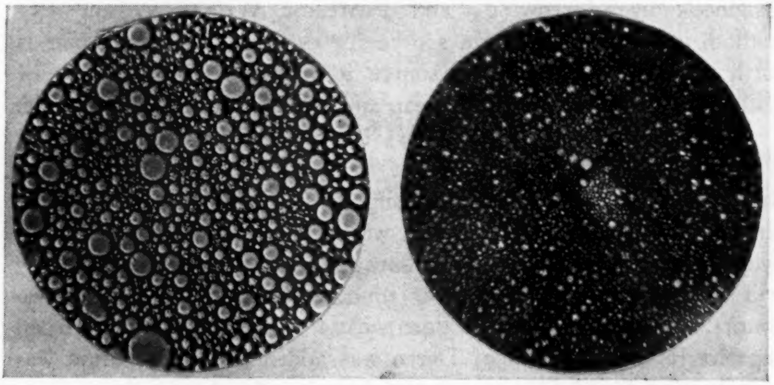

Fig. 3—The photomicrograph on the left is of an emulsion prepared by the use of the continental method. That on the right was prepared by the hand homogenizer.

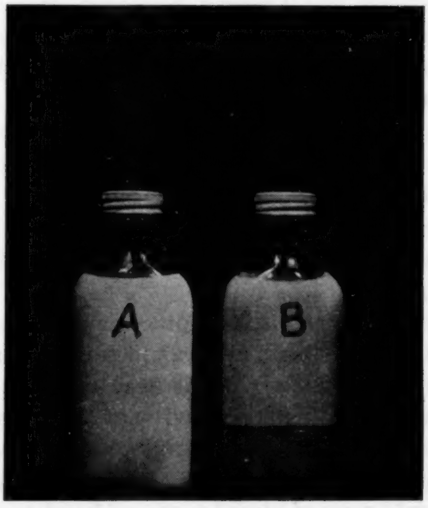

Fig. 4—The emulsions noted above, after standing 2 weeks. A. The homogenizer product. B. The continental method product.

METHOD OF SEPARATION OF LARGE AMOUNTS OF TYROSINE FROM CYSTINE

By Frederick R. Greenbaum, D. Sc.
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FOR experimental purposes I needed a good supply of cystine. The usual methods, such as the one described in Organic Synthesis by R. A. Gortner and W. F. Hoffmann (1), then the one in Biochemical Handbook by Morrow (2) and Professor Wright Wilson's (3) method, use human hair as a source for cystine. As human hair is not a very reliable and cheap source for commercial production of cystine, I decided to use other cheap and available sources for cystine.

According to Abderhalden (4) hog hair which is the cheapest type of animal hair, contains about 7.22 per cent. of cystine and the Armour Laboratories were kind enough to supply me with a five-pound sample of hog hair summer grade, which was defatted and dried. I hydrolized this hog hair with 4 liters of a 18 per cent. hydrochloric acid by heating with steam for nine working days (seventy-two hours). At the end of this time when the Biuret reaction turned negative, one-half pound of Darco was added and the solution was heated, stirred and filtered. In working up this batch, the various methods for isolation of cystine such as the one by Folin (5), Schmidt's (6) method and Organic Synthesis procedure (1) were tried, all of which gave cystine containing considerable amounts of tyrosine. It began to become obvious to me that hog hair contains a large amount of tyrosine in addition to the cystine. In order to determine the amount of cystine and tyrosine, the copper method by Philip A. Kober and K. Sugiura "On a Microchemical Method for the Determination of Alpha and Beta amino acids and Certain Derivatives in Proteolysis Blood and Urine" (7) and a later publication on "Improvement in the Copper Method for Estimating Amino Acids," by Philip Kober (8) was used.

The method consists in dissolving the sample containing the cystine and tyrosine in weak alkali, using 25 mg. of the sample and making up to 25 cc. total volume. To this was added 20 cc. of sodium borate, Sorensen's Buffer Solution, and 5 cc. of freshly precipitated cupric hydroxide containing 25-30 mg. of cupric hydroxide per 5 cc. Shake well, filter and pipette out 25 cc. portion of filtrate for tyrosine determination. Add 2 cc. of glacial acetic acid and 5 cc. of potassium

iodide solution. Iodine is liberated which on titration will give the tyrosine content.

To determine the cystine, the copper cystine precipitate and cupric hydroxide remaining on filter paper are washed thoroughly, dissolved in hydrochloric acid and after it has gone in solution, 2 gm. of potassium bicarbonate was added. When the cupric hydroxide was completely dissolved, the insoluble copper cystine complex was filtered, washed thoroughly and dissolved in acetic acid and the liberated iodine was titrated after the addition of potassium iodide and acetic acid. A 250/N sodium thiosulphate was used, which will give the amount of cystine. 1 cc. of 250/N sodium thiosulphate corresponds to 0.001448 gm. of tyrosine and 1 cc. of 250/N of sodium thiosulphate corresponds to 0.00096 gm. of cystine.

By means of this method the various fractions of cystine worked up by Folin's, Schmidt's, Organic Synthesis procedures were analyzed and gave the following results:

Sample of Cystine	Percentage of Cystine	Remarks
No. 2	89.36% Cystine	Fraction I—Organic Synthesis Method
No. 3	99.16% Cystine	Fraction I—Folin's Method
No. 4	82.44% Cystine	Fraction I—Schmidt's Method
No. 5	67.06% Cystine	Fraction II—Folin's Method
No. 6	53.05% Cystine	Fraction II—Schmidt's Method
No. 7	76.90% Cystine	Fraction II—Organic Synthesis Method
No. 8	39.25% Cystine	Fraction III—Folin's Method
No. 9	30.75% Cystine	Fraction III—Schmidt's Method
No. 10	71.82% Cystine	Fraction III—Organic Synthesis Method

The three fractions were obtained by precipitating the cystine with ammonium acetate, allowing to stand for twenty-four hours gave the first crop, Fraction I. After additional twenty-four hours the second crop was obtained, Fraction II, and on further standing for several days a third crop was obtained.

As can be seen from these figures, Folin's method gave a cystine almost 100 per cent. in the first crop, 67 per cent. in the second fraction and 39.2 per cent. of cystine in the third crop.

Schmidt's method gave 82 per cent. of cystine in the first crop, 53 per cent. of cystine in the second fraction and 30.75 per cent. of cystine in the third.

The Organic Synthesis method gave 89.4 per cent. of cystine in the first fraction, 76.9 per cent. in the second fraction, and 71.82 per cent. in the third fraction, so that the distribution of the cystine is more uniform in the three crops by the Organic Synthesis method and as the first crop is usually the smallest, the second and third are much larger, it seemed advisable to use the Organic Synthesis method.

To purify these fractions, the material was dissolved in the smallest amount of hydrochloric acid, heated to almost boiling, and a small amount of Darco was added and filtered. The filtrate should be colorless or faintly straw colored. If it still shows color, the treatment with Darco should be repeated. The cystine is precipitated from the clear solution by adding a saturated solution of sodium acetate (Organic Synthesis method) or by adding ammonium acetate to a pH of 4-4.5 according to J. Okuda and T. Kobayashi (9). When the cystine is precipitated it is allowed to stand for six hours, then filtered and washed with 100-200 cc. portions of hot distilled water which will remove the last traces of tyrosine.

Using this method of purification, I obtained no considerable improvement. I received on using the Organic Synthesis method 140 gm. of cystine which analyzed 72.6 per cent. and another fraction of 32 gm. of cystine with a content of 89.17 per cent. of cystine. In dissolving the 140 gm. of cystine (72.6 per cent.) in dilute hydrochloric acid and precipitating in the heat, I obtained three fractions.

Fraction I	32 gms	57.3% cystine
Fraction II	70 gms	73.6% cystine
Fraction III	24 gms	66.1% cystine

The other portion of 32 gm. of 89.17 per cent. of cystine when purified in the same fashion, gave only two fractions.

Fraction I	7 gms	97.24% cystine
Fraction II	21 gms	87.7% cystine

At one glance at these figures it is obvious that the purification of cystine as suggested in the classical methods for cystine is only applicable when the amount of tyrosine is small, which is the case when human hair is used. In other animal hair, however, particularly hog hair, a considerable amount of tyrosine is associated with the cystine and therefore on isolating the cystine, all fractions of it are more or less contaminated with tyrosine.

In order to recheck these findings I subjected the above fractions, with the exception of fraction I (97.2 per cent.), to another purification and obtained the following values:

From Fraction II—

21 gm. containing	87.7%	of cystine
First crop	92.66%	cystine
Second crop	77.30%	cystine
Third crop	55.92%	cystine
Fourth crop	50.00%	cystine

From Fraction II—

70 gm. containing	73.6%	of cystine
First crop	95.66%	cystine
Second crop	74.37%	cystine
Third crop	41.13%	cystine
Fourth crop	32.48%	cystine

From Fraction III—

24 gm. containing	66.1%	of cystine
First crop	89.94%	cystine
Second crop	53.04%	cystine
Third Crop	32.48%	cystine

It can be seen from these figures that while the first crops are purified and enriched in cystine, they did not give a 100 per cent. cystine, and the second, third and fourth crops represent a mixture of cystine with an appreciable quantity of tyrosine. It was therefore necessary to find

another method of purification which will permit to separate the large quantities of tyrosine from the cystine.

In some preliminary experiments I found that a precipitate of cystine with copper sulphate in ammonia forming copper cystine is completely insoluble in an excess of ammonia, while copper tyrosine is soluble in an excess of ammonia. On the basis of these observations I used 16.5 gms. of a fraction of cystine, which analyzed 55.9 per cent. of cystine. This fraction was suspended in 330 cc. of water, to which 40 cc. of hydrochloric acid (1:1) was added, which under mechanical stirring in the cold brought it in solution. Then 85 cc. of copper sulphate solution was added (1 gm. of copper sulphate in 5 cc. of water), which did not give a precipitate, and then 90 cc. of 26 per cent. of ammonia was added, a considerable excess, which precipitated the cystine copper and the tyrosine copper at first, but the excess of ammonia brought the copper tyrosine in solution. It was then filtered off, the copper cystine was resuspended in water, filtered, washed and suspended in 250 cc. of water with the filter paper and 50 cc. of hydrochloric acid (1:1) until all the copper cystine was dissolved and then filtered. The clear solution was heated to about 75 degrees C., then sodium sulphide solution was added under stirring, the temperature maintained at about 75 degrees C. Sodium sulphide was added until the filtrate did not give a copper precipitate with more Na_2S. The precipitated copper sulphide was filtered off and to the clear filtrate ammonium acetate was added until the pH was 4.5, which precipitated cystine. After several hours in the ice box it was filtered off, washed and dried and the analysis of this material was 39.21% of cystine, considerably lower than the cystine content of the fraction that I started with.

In another sample the copper cystine precipitate was thrown out, filtered, washed and dried and analyzed which gave 18.21 per cent. of copper instead of the theoretical 21.06 per cent. of copper. These findings seem to indicate that the copper destroys part of the cystine molecules and this confirms a private communication by Dr. M. X. Sullivan, who firmly believes that the presence of copper will destroy the cystine complex. This, of course, brought up immediately the question whether the microanalytical procedure by P. A. Kober and K. Sugiura, who are using minute quantities of copper for the determination of cystine will give accurate values as perhaps some destructive action of the copper might change the value of the cystine content.

However, on checking the figures by determining the nitrogen content, I found complete agreement, so that I feel that the copper method gives very reliable results.

The determination of nitrogen by Kjeldahl gave the following results in three samples:

Sample No. 4	11.27% Nitrogen
Sample No. 6	11.77% Nitrogen
Sample No. 10	11.21% Nitrogen

(Theoretical content of Nitrogen of Cystine, 11.66%.)

Calculating the nitrogen content from the results by copper method, I obtained:

Sample	No. 4	No. 10
Purity by copper method	92.6%	89.99%
Cystine calculated from nitrogen	90.08%	86.6%
Nitrogen calculated from copper method	11.37%	11.26%
Actual nitrogen found	11.27%	11.21%

From these figures it is evident that P. A. Kober's copper method gave very satisfactory analytical results. Apparently the amount of copper hydroxide used by this method is too small to have any destructive action.

I was therefore still confronted with the problem of separating large quantities of tyrosine from cystine. On going carefully over the literature, I noticed in Abderhalden, Handlexikon der Biochemischen Chemie, a reference to a method of purification of cystine by K. A. H. Moriner, who suggested to dissolve a mixture of cystine and tyrosine in hot 10 per cent. ammonia, then on cooling glacial acetic acid was added to weak alkaline reaction and tyrosine will now be precipitated. The tyrosine was filtered off and the filtrate was acidified with glacial acetic acid which threw down the cystine. Should the precipitate still give a positive Millon reaction, the procedure is repeated.

Using this information I took 95 gms. of cystine of a mixture of fractions of cystine containing 33 per cent., 45 per cent., 55 per cent., 75 per cent. and 83 per cent. of cystine. This was dissolved in 1425 cc. of water and to this was added 360 cc. of concentrated ammonia, heated to 75 degrees C., then Darco was added and filtered. Then I added after cooling at first 50 cc. of glacial acetic acid; no marked precipitation occurred. On the addition of another 30 cc. of glacial

acetic acid a precipitate was obtained which under the microscope showed crystals of a mixture of tyrosine and cystine. This gave 34 gms. of Fraction I, containing 45 per cent. of cystine. On the addition of 20 cc. of glacial acetic acid to the filtrate from Fraction I, a precipitate was obtained which under the microscope showed only crystals of cystine. This formed Fraction II of 17 gms. of a 100 per cent. cystine.

To the filtrate from Fraction II, 70 cc. of glacial acetic acid were added, which made it acid to litmus paper, and this was allowed to stand overnight and gave 24 gms. of Fraction II of 93 per cent. of cystine.

We have in this way obtained considerable improvement, Fraction II being 100 per cent. cystine which gave no Millon reaction; Fraction III, containing 93 per cent. of cystine, however, Fraction I was very low in cystine content.

In order to study this purification method I took a material which was high in cystine content. I used 18.5 gms. of a 91.1 per cent. cystine containing therefore actually 16.85 gms. of cystine and 14.0 gms. of a 92.6 per cent. of cystine containing 12.9 gms. of actual cystine. The two together gave 32.5 gms. of material containing 29.75 gms. of actual cystine.

The 32.5 gms. of substance were divided into two portions of 16.25 gms. each.

Portion I—16.25 gms. were dissolved in 250 cc. of hot 10 per cent. ammonia, Darco was added and filtered. On the addition of acetic acid it was fractionally precipitated and Fractions I and II were obtained.

Fraction I—3 gms. contained 99.4 per cent. cystine and gave a very faint Millon reaction.

Fraction II—8 gms. contained 89.9 per cent. cystine and gave a stronger Millon reaction.

Portion II—16.25 gms. of substance were suspended in 244 cc. of water heated to about 75 degrees C. and then the minimum amount of concentrated ammonia was added, filtered, cooled and fractionally precipitated with dilute hydrochloric acid which gave:

Fraction I—10 gms. contained 100 per cent. cystine and gave a negative Millon reaction.

Fraction II—2 gms. contained 47.66 per cent. cystine and gave a strong positive Millon reaction.

These experiments showed, first, it is better to use the minimum amount of ammonia instead of a 10 per cent. ammonia. Second, it is better to acidify with hydrochloric acid than with glacial acetic acid, but in both experiments the ammonia was used while the solutions were hot.

In another experiment the following changes were tried:

24 gms. of cystine containing 93 per cent. of cystine so that the actual content was 22.3 gms., were suspended in 450 cc. of water and 50 cc. of ammonia were added at room temperature, stirred for fifteen minutes, and then filtered off. The ammonia insoluble material was found to be by microscopic examination mostly tyrosine and formed Fraction I, 2 gms. containing only tyrosine. To the filtrate were added 160 cc. of hydrochloric acid (1:1) and methyl orange as indicator, mechanically stirred; this retained everything in solution, as the solution was now strongly acid. On addition of 62 cc. of 1:2 ammonia Fraction II was thrown out, which by microscopic examination seemed to contain only cystine crystals. Fraction II consisted of 17.5 gms. of 100 per cent. cystine, and on standing over night Fraction III, 2.5 gms. of a 38.5 per cent. of cystine was obtained. The recovery of the cystine of the total yield was 80 per cent.

Then a mixture of 26.5 gms. of material, which actually contained 25.1 gms. of cystine, was used. This was dissolved in 500 cc. of water, stirred mechanically, and 65 cc. of concentrated ammonia was added at room temperature, stirred for ten minutes, a little Darco was added and filtered. To this clear water white filtrate 150 cc. of hydrochloric acid (1:1) was added under stirring which precipitated cystine. This was filtered off and to the filtrate was added 26 cc. of 1:2 ammonia, which also gave cystine crystals and was filtered over the first precipitate of cystine, washed with water and with alcohol and on drying 21 gms. of Fraction I of 100 per cent. cystine was obtained with the Millon reaction entirely negative. This represents a recovery of 84 per cent. of the theoretical amount of cystine.

A mixture of various fractions of cystine of known and unknown cystine content was prepared by mixing them together in the following way:

9.5 gms. of substance of unknown cystine content
3.0 gms. of 82% cystine
9.5 gms. of 37% cystine
4.5 gms. of 45% cystine

together 26.5 gms. dissolved in 450 cc. of distilled water and 50 cc. of concentrated ammonia stirred for fifteen minutes and filtered. The ammonia insoluble residue according to the microscope is tyrosine and gave 13 gms. Fraction I of tyrosine.

To the filtrate was added 125 cc. of diluted hydrochloric acid (1:1) which made it distinctly acid and retained everything in solution, and 45 cc. of an ammonia solution (1:2) was slowly added under stirring. The solution was still in acid condition and very slowly precipitated on standing beautiful hexagonal crystals of cystine. It was filtered off and gave 7 gms. of Fraction II of 100 per cent. cystine with a Millon reaction entirely negative. On adding some more diluted ammonia, Fraction III of 4.5 gms. of tyrosine was precipitated. Total recovery, 24.5 gms. from 26.5 gms. of material.

In order to check the method I then took 25 gms. of 100 per cent. cystine and 25 gms. of 100 per cent. tyrosine, mixed them together and dissolved them in 900 cc. of water, added 100 cc. of concentrated ammonia to it and stirred for fifteen minutes. The ammonia insoluble portion, which represented pure tyrosine, was filtered off, dried and weighed and amounted to 19.7 gms. of tyrosine.

To the filtrate was added 25 cc. of (1:1) hydrochloric acid; 50 cc. of concentrated ammonia was diluted to 170 cc. and 90 cc. of this diluted ammonia was added, which gave a precipitate of cystine according to microscopic picture and the Millon test. Fraction II gave 23.8 gms. of 100 per cent. cystine, entirely negative for the Millon test.

To the filtrate of this 10 cc. of the above diluted ammonia was added, which gave a precipitate of 6 gms. of tyrosine, so that out of 25 gms. of cystine 23.8 gms. of cystine were recovered and out of 25 gms. of tyrosine 25.7 gms. of tyrosine were found, showing a very fair recovery of cystine amounting to 95.2 per cent. of the amount used.

These three fractions:

Fraction	I	19.7 gms. of tyrosin
Fraction	II	23.8 gms. of cystine
Fraction	III	6.0 gms. of tyrosine

were combined and dissolved in 900 cc. of water and 100 cc. of ammonia was added and stirred for fifteen minutes and filtered off. This gave Fraction I, 15.6 gms. of insoluble residue of tyrosine.

To the filtrate 250 cc. of hydrochloric acid (1:1) was added and 0.25 cc. of bromcresolgreen as indicator was used. Then 75 cc. of

(1 :2) ammonia was added and stirred, at this point cystine and tyrosine came down and the pH was found to be 4.2.

The solution was then made alkaline to about 7.8-8 to throw out everything so as to repeat this experiment. The experiment was actually carried out in this fashion, that at first to the acidified solution (1 :2) ammonia was added gradually and the following chart shows when tyrosine began to appear:

Amount of Ammonia used	50 cc.	55 cc.	60 cc.	65 cc.	68 cc.	70 cc.	72 cc.	75 cc.	80 cc.
Tyrosine	—	—	—	—	— +	+	+	+	+

This shows that there is a very definite point when tyrosine begins to appear.

In order to study this further 25 gms. of 100 per cent. cystine +25 gms. of 100 per cent. tyrosine were mixed and dissolved in 900 cc. of water and 50 cc. of ammonia was added, which cuts down the amount of ammonia previously used and it was stirred for three-quarters of an hour and filtered.

The insoluble residue consisting only of tyrosine weighed after drying 21.2 gms. To the filtrate was added 145 cc. of (1 :1) hydrochloric acid. To the perfectly clear solution was added slowly under stirring ammonia (1 :2) in small portions starting out at first by adding 45 cc. of ammonia and checking the pH with methyviolet. After the addition of 45 cc. of ammonia (1 :2) the pH was 1.2 cystine positive tyrosine negative.

Time for stirring	Amount of Ammonia added	pH	Cystine	Tyrosine
20 minutes	3 cc.		—	—
30 "	2 cc.	Less 1.04	±	—
15 "	5 cc.	" 1.04	+ +	—
15 "	5 cc.	" 1.04	+ + +	—
15 "	5 cc.	About 1.04	+ + + +	—
15 "	5 cc.	" 1.17	+ + + +	—
15 "	5 cc.	" 1.17	+ + + +	—
15 "	5 cc.	" 1.42	+ + + +	—
15 "	5 cc.	" 1.92	+ + + +	—
	Stood overnight			
10 "	5 cc.	About 2.27	+ + +	+

From this experiment one can see that the isolation point of a mixture of cystine and tyrosine is very much more on the acid side than investigators have heretofore believed it to be, who recommend a pH from 4 to 4.5 and some even as high as a pH of 6.

The next experiment intended to study the influence of time of stirring with ammonia solution.

25 gms. of cystine
25 gms. of tyrosine

mixed and suspended in 900 cc. of water and 50 cc. of concentrated ammonia allowed to stir for one and one-half hours, then filtered off gave: Fraction I insoluble in ammonia amounting to 23 gms. of tyrosine. To the filtrate was added 140 cc. of hydrochloric acid (1:1) and allowed to stand over night. At a pH of 1.42 no tyrosine appeared. Then 68 cc. of (1:2) ammonia was added which brought the pH to 1.92, then filtered off, which gave Fraction II; no tyrosine was present; this amounted to 23.5 gms. or 94 per cent. of recovered 100 per cent. cystine.

On standing over night Fraction III was obtained, consisting of 2.5 gms. of tyrosine giving a recovery of slightly over 100 per cent. of tyrosine.

From the previous experiment which yielded 23 gms. of tyrosine, 23.5 gms. of cystine and on standing over night 2.5 gms. of tyrosine. Fraction I and III were combined giving 25.5 gms. of tyrosine, and the separation method was tried with the idea in mind to isolate the very small amount of cystine (0.5 gms.) which was contained in the 25.5 gms. of tyrosine. On suspending the 25.5 gms. of tyrosine in 450 cc. of water and on the addition of 25 cc. of concentrated ammonia, it was stirred for one hour and a half and filtered off and gave the ammonia insoluble portion, Fraction I, 19.5 gms. of tyrosine and then 72 cc. of hydrochloric acid (1:1) were added and allowed to stand over night, which gave 5.5 gms. of tyrosine as Fraction II, showing that such small amounts as 0.5 gms. of cystine in 25.5 gms. of tyrosine cannot be recovered.

The following table shows the results obtained in a number of experiments with this method of separation of tyrosine from cystine:

Amount of Crude Cystine Used	First Crop Insoluble in Ammonia (Tyrosine)	Second Crop (100% Cystine)	Third Crop	Amount of Tyrosine Recovered	Amount of 100% Cystine Recovered	Per Cent. of Cystine Obtained
26.5 gms. 35 gms. of Cystine Content	13 gms.	7 gms.	4.5 gms.	17.5 gms.	7 gms.	88%
24 gms. Actual Cystine Content 22.3 gms.	2 gms.	17.5 gms.	2.5 gms.	4.5 gms.	17.5 gms.	80%
26.5 gms. Actually Contained 25.1 gms. of Cystine	—	21 gms.	—	—	21 gms.	84%
50 gms. of a Mixture of 25 gms. of Cystine C. P. 25 gms. of Tyrosine C. P.	19.7 gms.	23.8 gms.	7 gms.	26.5 gms.	23.8 gms.	95.2%
25 gms. of Tyrosine 25 gms. of Cystine	23 gms.	23.5 gms.	2.5 gms.	25.5 gms.	23.5 gms.	94.0%

Summary

(1) A method for the separation of large amounts of tyrosine from cystine is presented.

(2) Usually from 92 to 94 per cent. of cystine may be recovered.

(3) The point of isolation of a mixture of cystine and large amounts of tyrosine is at a pH of 1.72 to 2.0.

(4) The method is unsuitable and fails if very small amounts of cystine are mixed with very large amounts of tyrosine.

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ISOLATION OF A VIRULENT AND HIGHLY TOXIGENIC STRAIN OF C. DIPHTHERIÆ

By George F. Leonard and August Holm †

WHEN newly isolated strains of C. diphtheriæ are investigated in respect to their ability to produce toxin in vitro, it is very often found that they are poor toxin producers although occasionally of high virulence. It was therefore thought of interest to report the isolation of a strain of C. diphtheriæ with an ability to produce toxin in vitro which apparently equals in every way that produced by the Park-Williams No. 8 strain, and also of a high virulence.

The usual classification of the different strains of C. diphtheriæ according to agglutination does not give information respecting possible variation in biochemical activity. In fact, it has until lately been taken for granted that all true diphtheria organisms attacked growth substrate in essentially the same manner as evidenced by the identity of the toxin produced. However, the isolation in England by Anderson and co-workers (1) and others (2) of starch and glycogen fermenting strains, with a possible connection with certain clinical aspects of the disease, has stressed the importance of an investigation of newly isolated organisms in respect to their fermentative activity and other growth characteristics.

The English workers have divided three variants of C. diphtheriæ into three types which they have designated *gravis, mitis* and *intermediate*. C. diphtheriæ gravis ferments glycogen and starch and is non-hemolytic. C. diphtheriæ mitis ferments neither glycogen nor starch and is hemolytic, and the intermediate type ferments neither glycogen nor starch. Colony formation on chocolate-tellurite medium is also a differential feature of these types.

History

R. D., male, 25 years, complained of sore throat, which on examination three days later showed an extensive membrane formation, and had a very fœtid odor. Temperature at that time was 100.2 degrees F. and throat culture was positive for C. diphtheriae. Twenty thousand units of diphtheria antitoxin were then injected, after which there was rapid clearing of the membrane, and an uneventful recovery.

† From the Biological Laboratories, E. R. Squibb & Sons, New Brunswick, N. J.

Culture Study

The organism was isolated by plating in the usual manner, and the pure culture transferred to semi-synthetic medium (3) on which it formed a pellicle in twenty-four hours, while the medium remained clear. On shaking, the pellicle broke up into fine granular particles which readily settled to the bottom of the tube leaving the supernatant liquid transparent and clear. This growth is characteristic for the intermediate type. The strain isolated from this case was designated No. 487.

Transplanted on the same medium with 0.2 per cent. dextrose, the toxin formed in a slanted test tube in eight days contained 4 Lf units per cubic centimeter, while the Park-Williams No. 8 strain produced toxin of 5 Lf units under the same conditions. Experimental bottles of culture media under the conditions of large scale toxin production (4) when planted with Strain No. 487, contained 32 Lf units per cubic centimeter after ten days' incubation, or the same strength of toxin as produced by the Park-Williams No. 8 Strain. The curve of toxin production under these conditions may be seen from Figure 1. Animal tests showed the m. l. d. of both toxins from Strains No. 487 and from Park-Williams No. 8, to be 0.001 cc. and the L+ value 0.04 cc. The plating of Strain No. 487 on tellurite agar produced colonies of the intermediate type, while fermentation tests disclosed inability to ferment starch.

The antigenicity of a toxoid prepared from toxin produced by Strain No. 487 was equal to the antigenic activity of the toxin from Park-Williams No. 8 strain, when tested on guinea pigs.

The newly isolated strain showed the characteristics of the intermediate type, and it was found that the Park-Williams No. 8 strain could also be classified as this type, which is in accordance with the findings of Anderson and co-workers (1).

Summary

A virulent strain of C. diphtheriæ was recently isolated which was highly toxigenic, even in semi-synthetic medium, and which was comparable in all respects to Park-Williams No. 8 strain.

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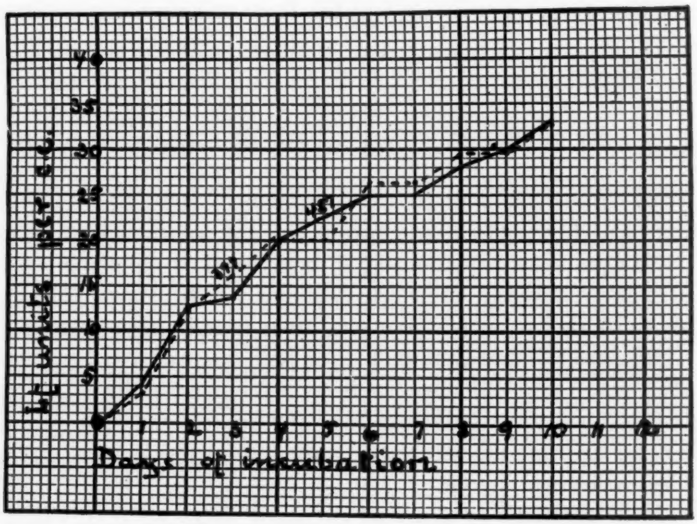
Figure I.



No. 397 is a Park-Williams No. 8 strain.
No. 487 is the newly isolated organism.

THE OILS OF ARTEMISIA RIGIDA (NUTT.) GRAY

By G. Norin and R. L. McMurray

THE scab-land sagebrush was described first by Nuttall (1) in 1841 as *Artemisia trifida* var. *rigida,* but was set apart as the species *Artemisia rigida* (Nutt.) Gray by Asa Gray (2) in 1883.

This plant is restricted in its range to Eastern Washington and Oregon and adjacent regions and is found mostly on thin, rocky soil of bluffs and ridges. It is a low shrub about two to six decimeters high, with a brown, twisted, fibrous stem and shrubby branches. The leaves are sessile, flattened spatulate, about 1.5 to 4 centimeters long, 1 millimeter wide, and parted into 3 to 5 narrowly linear divisions. The inflorescence is a leafy spike 2 to 15 centimeters long and less than 1 centimeter wide, exclusive of the leaves, and bearing numerous yellow disc flowers. The plant aroused curiosity as a possible source of santonin because of its being a member of the santonin-producing genus of plants, *Artemisia,* and because of its late blooming time—September. The following work arose from this primary investigation of the plant. (3)

The Volatile Oil

The blooming tops of *Artemisia rigida* (Nutt.) Gray, were gathered October 7, 1933, on the high bluffs above the Snake River in Whitman County, Washington, the refuse culled out, dried, and ground to approximately a No. 20 powder for analytical determinations. The ground material was stored in air-tight containers. October 5, 1934, 8.4 kilograms of this stored material (air dried) was available for the production of the volatile oil. It was subjected to steam distillation and yielded 43.7 Gm. of volatile oil. The stereoptene was separated by chilling the volatile oil to 15° C. and suction filtering. The stereoptene amounted to 12.2 Gm. and the oleoptene to 31.5 Gm. Thus, the blooming tops of *Artemisia rigida* (Nutt.) Gray yielded 0.56 per cent. of a volatile oil, of which 27.92 per cent. were stereoptene and 72.08 per cent. was oleoptene at 15° C.

The oleoptene was amber to yellowish in color, becoming deeper colored with age; odor pungent and somewhat camphoraceous; taste warm, persistent and aromatic; feel turpentine-like. It was acid to litmus paper. It was miscible with absolute alcohol, 95 per cent. alcohol, acetone, glacial acetic acid, chloroform, ether and petroleum

ether; immiscible with carbon disulfide. The following constants were obtained on the oleoptene:

Specific Gravity, 25°/25° C.	0.9367
Optical Rotation, 100 mm. tube, 25° C.	—15.68°
Specific Rotation, 25° C.	—16.75°
Refractive Index, 25° C.	1.4674
Acid Value	3.63
Ester Value	19.46
Saponification Value	23.09

The Fatty Oil

After the removal of the volatile oil by steam distillation, the material was dried and packed in a percolator. It was subjected to extraction with alcohol (95%), using the alcohol continuously recovered from the extract. After the rate of extraction was negligible, the excess alcohol was recovered from the extract.

The extract was then shaken out successively with petroleum ether to remove the fatty portion. From this the petroleum ether was recovered by distillation and the fatty oil redissolved in anhydrous-ether. The anhydrous-ether extract was then further dehydrated by the use of anhydrous sodium sulphate, filtered, the ether removed and heated on a steam bath to constant weight. The fatty oil amounted to 158 Gm. or 1.88 per cent. of the air-dried material.

The fatty oil was viscid and very dark green in color. The following constants were determined for this fatty oil of *Artemisia rigida* (Nutt.) Gray.

Specific Gravity 40°/40° C.	0.9945
Refractive Index 40° C.	1.4968
Acid Value	36.68
Ester Value	91.64
Saponification Value	128.32
Iodine Value	58.71

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SCIENTIFIC AND TECHNICAL ABSTRACTS

Compiled by Arthur Osol, Ph. D.

New Drug Extraction Processes. Francis Chilson. *Drug and Cosmetic Industry, 36,* 37 (1935). In describing vacuum extraction processes for drugs the author calls attention to the fact that maceration and gravity percolation methods are inefficient and wasteful and that the results are variable. Thus, a chemist using a test quantity of drug, obtains 5 to 200 per cent. more extract than can be obtained from the same lot of drug extracted under plant conditions. In gravity percolation methods, the labor of grinding the drug, loading the percolators, carrying away the percolate in small containers, recovering the solvent in the spent marc, etc., is accepted as an unavoidable part of the operation.

Gravity percolation necessitates very fine grinding of hard, woody drugs and coarse grinding of soft or friable drugs, while in vacuum percolation it is impractical to grind finer than ten or twelve mesh. In the case of gravity percolation the active principle is more or less dissolved out of the marc by saturation. In vacuum extraction processes, the menstruum is driven through the cells of the marc by impregnation. This is accomplished by putting the ground drug into the extractor, adding the menstruum, mixing for a few minutes and then drawing a high vacuum on the extractor. This is maintained for ten or fifteen minutes and its effect is to draw all the air out of the cells of the drug. The vacuum is then broken and the menstruum instantly saturates the drug particles and replaces the air withdrawn. The power of this phenomenon is such that alcohol can be driven through a board in a few minutes. The percolate is then run off and a series of washes are put through the drug in the manner described above. After collection in glass-lined tanks the percolates are continuously drawn into a vacuum still at a rate of speed approximately equivalent to the concentration speed. Virtually all of the alcohol is saved and the strength of the extracts obtained from sensitive drugs has been increased from 50 to more than 100 per cent. compared to gravity percolation and atmospheric concentration methods. The time cycle is about three days for extraction, concentration and solvent recovery.

The author is now working upon a method whereby one wash would suffice for the extraction of the drug. In this process 95 per cent. of the solvent put on at the beginning of the operation would be removed and the quantity of extract remaining would be very small. The method embraces the idea of using a vacuum extractor with an agitator and a continuous centrifugal to separate the percolate from the marc.

Sensitive Reaction for Boric Acid. A. S. Komarovski and N. S. Poluektov. *Mikrochemie, 14,* 317 (1934). A drop of the solution under test, made slightly alkaline, is evaporated to dryness in a porcelain dish and two or three drops of a 0.005 per cent. solution of p-nitrobenzeneazochromotropic acid (Chromtrope 2B) added. In the presence of as little as two parts per million of boric acid the color changes from bluish violet to greenish blue. In the presence of oxidizing anions a rose or yellow precipitate is produced in which case a drop of the solution should be heated with solid hydrazine sulfate until fumes of SO_3 are evolved, before adding the reagent. If fluorine is present the acidified solution under test is warmed with SiO_2 to remove the fluorine as SiF_4. The method is particularly suited for the detection of boron in minerals.

Quinaldinic Acid as a Micro-Reagent. I. Estimation of Zinc and Its Separation From Manganese. P. R. Rây and M. K. Bose. *Mikrochemie, 17,* 11 (1935). A macro-method for the estimation of zinc in the presence of manganese, magnesium, alkaline earths, phosphoric and arsenic acids as quinaldinate has recently been described by Rây and Bose (*Zeit. f. anal. Chem., 95,* 400, 1934). The crystalline character of the precipitate, low percentage content of zinc (15.29) in the compound, and the accuracy of the results obtained by the macro-method suggested a micro-method for the same. The following procedure was used in the determination of zinc in potassium zinc sulphate. About 0.6 to 7 mgm. of the salt (corresponding to 0.086 to 1 mgm. of zinc) was weighed into a microbeaker and dissolved in about 1 to 1.5 cc. of water. The solution was acidified with one or two small drops (0.02 to 0.04 cc.) of glacial

acetic acid and then heated for a minute in its stand on a boiling water bath. The zinc was precipitated from the hot solution as quinaldinate by adding, drop by drop, a solution of sodium quinaldinate (equivalent to 1 gm. of quinaldinic acid per 100 cc.), frequently rotating the beaker during the addition of the reagent. An excess of 0.2 to 0.25 cc. of reagent is added (0.2 to 1 cc. is the quantity theoretically required). After heating again for a minute, the precipitate is allowed to settle and collect at one side of the beaker. The supernatant liquid is drawn off through an Emich asbestos mat filter stick, and the precipitate sucked as dry as possible. After washing five or six times with 0.5 to 1.0 cc. of hot water, the beaker with the precipitate and the filter stick is first heated by placing it for a minute or two on a boiling water bath to remove all adhering water, and then finally dried for ten minutes in a current of air at 125 degrees C. in a Benedetti-Pichler drying apparatus. After wiping the beaker with a moist flannel and dry chamois respectively, it is weighed on a micro-balance. Excellent results were obtained in the analysis of potassium zinc sulphate and zinc sulphate alone and in the presence of manganese, magnesium, alkaline earths and phosphoric acid.

Zinc has also been estimated micro-chemically in the presence of iron, aluminum, beryllium, titanium and uranium, the precipitation of the latter metals being prevented by the addition of alkaline tartrate solution.

Quinaldinic Acid as a Micro-Reagent. II. Estimation of Copper and Its Separation From Cadmium, Manganese, Nickel, Cobalt, etc. P. R. Rây and J. Gupta. *Mikrochemie, 17,* 14 (1935). Quinaldinic acid precipitates copper from hot acid solutions as a heavy, crystalline, green precipitate, absolutely insoluble in hot water. The precipitate is washed with hot water, dried at 125 degrees C. and weighed as such (copper content 14.96 per cent.). The method separates copper from cadmium, lead, manganese, nickel and cobalt, as well as from phosphoric, arsenious and arsenic acids. The amount of copper taken for analysis should be within 0.1 to 1 mgm. as amounts greater than this produce large quantities of precipitate which are difficult to filter.

Cholesterol in Cosmetics. E. A. Lum. *Pharm. Journ. 134,* 291 (1935). The note gives information on the use of cholesterol and lecithin in creams which are stated to keep the skin of the user free from wrinkles and to remove these signs of age. Pure crystalline cholesterol and vegetable lecithin are used. It has been shown recently that cholesterol and lecithin occur in the natural oily exudation of the skin, preventing it from drying, and there are, therefore, good grounds for the properties claimed for them. It is necessary to include readily absorbable oils and fats in the cream, otherwise the cholesterol and lecithin become useless. With these facts in mind, the following formula is suggested:

Almond Oil	50.0
Anhydrous Lanolin	6.5
White Beeswax	13.0
Water	26.0
Borax	1.0
Cholesterol	1.0
Lecithin	1.5
Sodium Benzoate, 1.0; or Nipagen	0.2

Dissolve the cholesterol and lecithin in the mixed and melted wax, anhydrous lanolin and oil. Filter. Dissolve the borax and the preservative in the water, previously heated to about 75 degrees C. Allow the mixed fats to cool to this temperature, and add to them the aqueous solution in a constant stream, while stirring, and continue stirring until the product congeals. Perfume, if any is desired, should be added just before congelation. The resultant cream is of a very smooth consistency and has a light chocolate brown color. The method of application is to massage a little of the cream into the skin each night for at least five minutes. No growth of facial hair is produced by continued use of the cream.

Aconitine in Aconite Root. Assoc. Official Agr. Chem. *Journ. Assoc. Official Agr. Chem., 18,* 84 (1935). The following tentative method for the detection of aconitine in aconite root was adopted. Crush and macerate aconite root with 15 cc. of distilled water, transfer to a Mojonnier extraction tube, add 5 cc. of 10 per cent. NH_4OH and extract two or three times with 15 cc. portions of ether. Transfer

the ethereal extract to a separator, wash with water and extract with 2 or 3 cc. portions of 0.02 N H_2SO_4 until the aqueous layer remains acid to methyl red. Test the slightly acid aqueous layer for aconitine by the following method: In a small test tube add one or two drops of 5 per cent. Na_2CO_3 to 1 or 2 cc. of the solution to be tested, heat to 60 degrees C., stir, cool and transfer a few drops of the liquid to a micro-slide and examine the crystals. Irregular hexagonal plates are formed by aconitine. Most characteristic crystals of aconitine are formed in solutions of a strength of 1:1000 or less.

Tetrachloroethylene in Mixtures. Assoc. Official Agr. Chem. *Journ. Assoc. Official Agr. Chem., 18,* 84 (1935). The following method for the determination of tetrachloroethylene in mixtures containing liquid petrolatum and oleoresin of aspidium was adopted as a tentative method. In a weighed 125 cc. cork-stoppered Erlenmeyer flask, place sufficient sample to give the equivalent of about 0.16 gram of tetrachloroethylene and weigh. Add 10 cc. of xylene and two grams of sodium reagent, connect flask to a reflux condenser and heat on a hot plate to boiling. Add 1 cc. of amyl alcohol through the condenser, reflux gently for two hours and at intervals add 1 cc. portions of amyl alcohol until 5 cc. have been added. Disconnect flask, cool, destroy excess of sodium with 20 cc. of water, acidify with nitric acid and transfer mixture to separator. Shake xylene layer with three 10 cc. portions of water, filter the acid, aqueous solutions into a 200 cc. volumetric flask, add 50 cc. of 0.1 N $AgNO_3$ solution and dilute to 200 cc. After shaking thoroughly pour the mixture through a dry filter, discard the first 20 cc. of filtrate and titrate a 100 cc. aliquot with 0.05 N NH_4CNS, using ferric alum as indicator. Make a blank test for chloride. Each cc. of 0.1 N $AgNO_3$ is equivalent to 0.004146 gram of C_2Cl_4.

Mercury in Mercurial Ointment. Assoc. Official Agr. Chem. *Journ. Assoc. Official Agr. Chem., 18,* 85 (1935). Weigh one gram of the thoroughly mixed ointment into an Erlenmeyer flask, add 20 cc. of water and 20 cc. of HNO_3 and heat gently over a small flame until red fumes cease to evolve. Cool and decant the aqueous solution into a separator. Wash the ointment base with 50 cc. of boiling water, cool, and decant into the separator. Repeat the washing until

all of the mercury is removed. Shake the combined solutions in the separator with 50 cc. of ether, transfer the aqueous solution to an Erlenmeyer flask, wash the ether three times with 10 cc. portions of water until the mercury is removed and add the washings to the flask. Add 3 cc. of ferric alum test solution (U. S. P.) and titrate with 0.1 N NH_4CNS. Each cc. of 0.1 N NH_4CNS is equivalent to 0.01003 gram of mercury.

Detection of Homatropine, Hyoscyamine and Scopolamine. Assoc. Official Agr. Chem. *Journ. Assoc. Official Agr. Chem., 18,* 85 (1935). Microchemical methods for the detection of these alkaloids are given, the test being carried out by adding a drop of gold chloride reagent (1 gram in 20 cc. of water) to a drop of alkaloidal solution on a clean glass slide and examining the resulting crystals under a microscope with 100-150 magnification. Homatropine forms green-gold blades, often with pointed ends and united in pairs, the surfaces appearing etched on long standing. Scopolamine produces clusters of pale yellow, transparent blades, with coarse saw-tooth edges forming immediately on shaking the slide to stir the solutions. Hyoscyamine forms thin, transparent, nearly colorless, irregular plates, often curved. The tests should be compared with controls prepared by dissolving one milligram of pure alkaloid in two drops of water.

Detection of Amidopyrine and Dinitrophenol. Assoc. Official Agr. Chem. *Journ. Assoc. Official Agr. Chem., 18,* 86 (1935). After separating the compound in pure form by the use of suitable solvents, the portion suspected as being amidopyrine is dissolved in water and portions of this solution tested with $HgCl_2$ reagent (5 grams in 100 cc. of water) and with Marme's reagent (3 grams of CdI_2 in 18 cc. of water containing 6 grams of KI). The former produces long, slender radiating crystals, often curved, while the latter produces groups of spiny branches when observed on a glass slide under a microscope. With Marme's reagent one drop of 5 per cent. HCl should also be added.

The sample which is believed to be dinitrophenol is dissolved in a small quantity of 0.1 N NaOH, 1 per cent. HCl added, and the mixture observed under a microscope. Dinitrophenol forms plates with four branches. In more dilute solutions single rectangular plates are formed. Comparisons with controls should be made.

SOLID EXTRACTS

By Ivor Griffith, Ph. M., Sc. D.

In all seriousness, the Council on Pharmacy and Chemistry of the American Medical Association pronounces the malediction upon aspirin, charging it with carrying a poison sting, quite as venomous as that of its first syllable.

And, state the solons in Chicago, it should not be admitted to the medicine chest except on the say so of the wise old family doctor who knows the dangers lurking in its malignant molecules.

Well, well! and so aspirin goes to join the innumerable throng of drugs discredited.

Yet the public will persist in the purchase of it and the counters of the V and X continue to enjoy its transient company, for it is a strange commentary upon these medical interdictions, that old John Public scarcely heeds them and listens only to his own convenience.

And who is there to say that the public proving ground and the telling tests of time are not fairly adequate after all?

Dusk obliterates the rose,
Night is so unkind to color,
But when dew, at morning, blows
Every rose is richer, fuller.

Which came to our mind upon reading, the other day, of a totally blind man, whose sense of touch has been so sharpened by his loss of vision that he can actually differentiate color in textiles with the aid only of his finger tips.

Such an achievement is not just a cause for wonder, but an invitation as well as a challenge to those who study the plight of the blind with a view to keening the rest of their senses.

"A pint's a pound the world around" is another of those unfortunate statements, richer in rhyme than in reason. To realize the

enormity of the error involved in the lie-lilting line, one only has to recall that a pint of ether weighs only about three-quarters of a pound and a pint of mercury almost fourteen pounds.

Words are consistently seeping into our vocabulary—either with malice aforethought (e. g., halitosis) or else from natural sources and through natural forces. Take for instance the coinage just cast for that conglomerate of breakfast and lunch, for those too lazy to rise for the first meal and too hungry to wait for the mid-day meal. *Brunch*—is the word!

Then along comes Kansas out of the dust, long enough to christen its mixture of snow and dust—*snust!*

But worst of all is the minting, by a New York columnist, of a new name for the lunch serving drug store. We've heard it called the "drudge store" by the apprentice lad—but this is the first time we've ever *seen* it called the *Drunch* store!

The nursery rhyme used to teach us that we are made of "scissors and snails and puppy dogs' tails," or "sugar and spice and everything nice." Not so with chemistry, however. Here is the total humorous composition of the creature called man: Fat enough for seven bars of soap, sugar enough to sweeten two cups of Child's coffee, lime enough to whitewash a cellar around the bottles, phosphorous enough to make four boxes of matches, magnesia as much as the druggist sells for a nickel—and that is not much—iodine enough to paint a pimple, and sulphur enough to rid a dog of fleas. Many items in this estimate are left largely to the imagination, such as the size of the dog and the number of his tormentors, but the total cost is given, a la Lit, as ninety-eight cents, which is neither expensive nor calculated to foster conceit.

Lady Nicotine has been blamed for most of tobacco's mischief, yet wicked as the lady is, her's is not always the fault.

Skin tests, such as the diagnostic tests for hay fever, have developed the fact that "one out of three have *it*"—*it* being a protein reaction or sensitiveness to tobacco.

Dr. Harkavy, of New York City, who is an allergic specialist, reports that his work has not gone far enough to state definitely how big a part tobacco sensitivity plays in the production of a certain type of heart and blood vessel disease. He thinks that if persons who are sensitive smoke enough they may get the disease. Many persons, however, show sensitiveness to tobacco without any disease symptoms.

Still, all of these indictments seem unlikely to achieve to any "cigareticence"!

That genealogical table in Genesis indicates that the ancient scribes valued well the line of descent, and knew how the child must, to some degree, be as the parents were. But here is a striking academic genealogy that one rarely meets in written record.

J. U. Neff, the famous chemist, was a pupil of von Bayer, von Bayer was a pupil of Kekulé, Kekulé was a pupil of Liebig, and Liebig was a pupil of Gay-Lussac. What a heritage! Neff's studies on tautomerism, keto-enol forms and bivalvent carbon paved the way for much that has since developed great value, both scientific and commercial, in the field of dye chemistry. The fund of theoretic and practical information based on these researches is invaluable and yet Neff probably profited not one penny by it. He no doubt felt well repaid by his success in achievement. Science is the richer because of his labors and industry is able to operate the better because of his explanations of keto-enol tautomerism.

The entire volume of circulating blood, which about half fills an ordinary bucket, contains only a small teaspoonful (from 4 to 6 gms.) of sugar and a heaping tablespoonful (32 gms.) of salt, ordinary—common—salt. When we consider the minute variations in the sugar content that the modern chemist can measure in a few drops of blood, we gain added respect for the science of quantitative analysis. The

iodine in the entire blood amounts to but .001 gm., or an average dose of atropin. When the physiologist tells us that adrenalin can be detected by biologic methods in a dilution of 1:330,000,000, it means far less than to say that it is equivalent to diluting "a small glass of whiskey (10 cc.)"—a very small glass, that—into the contents of 1320 city street sprinkling carts, which would form a procession about six miles long.

Heavy Water Molecules Trace Water in Body

When you take a drink of water half of it is still in the body after nine days. And the average time a water molecule stays in the body is thirteen days.

This is the summary of investigations making use of *heavy water* for physiological studies of the water content of the human body developed by Prof. Georg von Hevesy and E. Hofer, of the University of Freiburg in Germany.

Because heavy water molecules can be distinguished by physical tests, although inseparable chemically from ordinary water, they can act as "tracers" in studying how the body eliminates water. Previously physiologists have never been able to make exact tests of how long the water in any particular "drink" stayed in the body.

The scientists were able to make estimates of the amount of water in the body. Their value of from 59 to 67 per cent. is in fair agreement with known data.—*Science News Letter.*

New Mold Makes Sarcolactic Acid

Another mold has been harnessed and put to work by chemists in the U. S. Department of Agriculture. This one is a species of Rhizopus and a relative of the common bread mold. When properly fed and cared for this Rhizopus produces sarcolactic acid, a component part of ordinary lactic acid, the acid found in sour milk. Work which led to the discovery of this mold was recently described before the American Chemical Society in New York in a paper by G. E. Ward, L. B. Lockwood, O. E. May, and H. T. Herrick, of the Bureau of Chemistry and Soils.

Sarcolactic acid derives its name from the fact that it was originally prepared in 1808 from animal flesh, which is still one of the principal sources. It is found in small quantities in the human body, and plays an important part in human metabolism—the process by which the body converts food into fuel and energy. Because of limited sources heretofore available sarcolactic acid has been very hard to get in quantity in pure form, and difficulties encountered in its preparation have held the price near a dollar a gram. With the new molds at work indications are that this price can be materially cut, thus making pure sarcolactic acid generally obtainable for physiological and industrial investigations.

Heretofore the only lactic acid produced industrially has been the inactive form, which is manufactured by the bacterial fermentation of starch. The sarcolactic acid which has been made in the laboratory up to the present time has usually contained impurities. The new process now being developed in the Department gives relatively high yields of a pure product.

Discovery of the mold that makes sarcolactic acid is an outgrowth of a study, begun in 1926, of the application of molds to the utilization of farm products. Other molds have been found for making gluconic and kojic acids.

Correspondence

Ivor Griffith, Editor
AMERICAN JOURNAL OF PHARMACY
43rd Street & Kingsessing Avenue
Philadelphia, Pa.

March 25th, 1935.

Dear Sir:

Recently I received a letter from the Commissioner of Narcotics calling attention to an item which appeared in the AMERICAN JOURNAL OF PHARMACY of September 1934, relative to the Kabay process for making morphine from so-called poppy straw. The Commissioner refers to an error in the percentages of yields of morphine and codeine from the raw material used, it being represented that the statements of yields were ten times too large. It was argued, the communication goes on to say, that this exaggerated statement of yields might inspire the growth of the opium poppy in the United States with the intention of extracting alkaloids by the Kabay method.

The Commissioner's further comments are quoted below:—

"This matter was taken up with the State Department to obtain its views as to the possibility of correcting the apparently erroneous impression of yields by the Kabay method, by making available the actual yields as ascertained from an authoritative source. I am now authorized to advise that in accordance with a League of Nations document, the Hungarian Government in a memorandum in regard to the Alkaloida Company (Kabay) process stated last autumn that 'The results of manufacture give an average morphine base of 0.8 per mille and codeine base of 0.08-0.04 per mille.' I therefore suggest that you, as the representative of the domestic manufacturers, communicate this correction to the publishers of the AMERICAN JOURNAL OF PHARMACY and of Industrial and Engineering Chemistry, with the recommendation that they publish the correct information in order to correct any erroneous impression that may have been obtained by their subscribers from the former articles on the Kabay process."

Commending the foregoing to your attention, I am

Cordially yours,

CARSON P. FRAILEY,

Executive Vice-President American Drug Manufacturers Association.